Supplemental material

Cadmium Impairs Albumin Reabsorption by Downregulating Megalin and ClC5 Channels in Renal Proximal Tubule Cells

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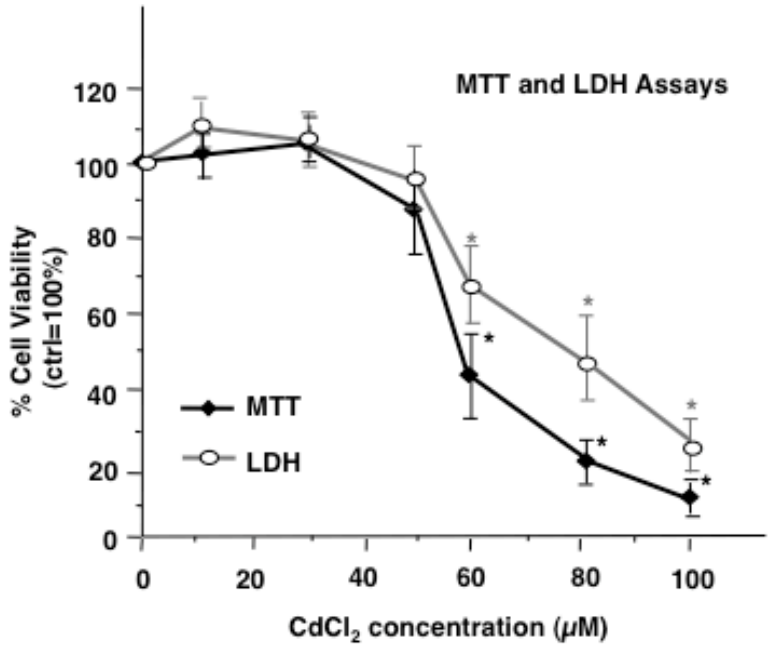
Supplemental Material, Table 1. Primers used for the Real-Time quantitative PCR analysis

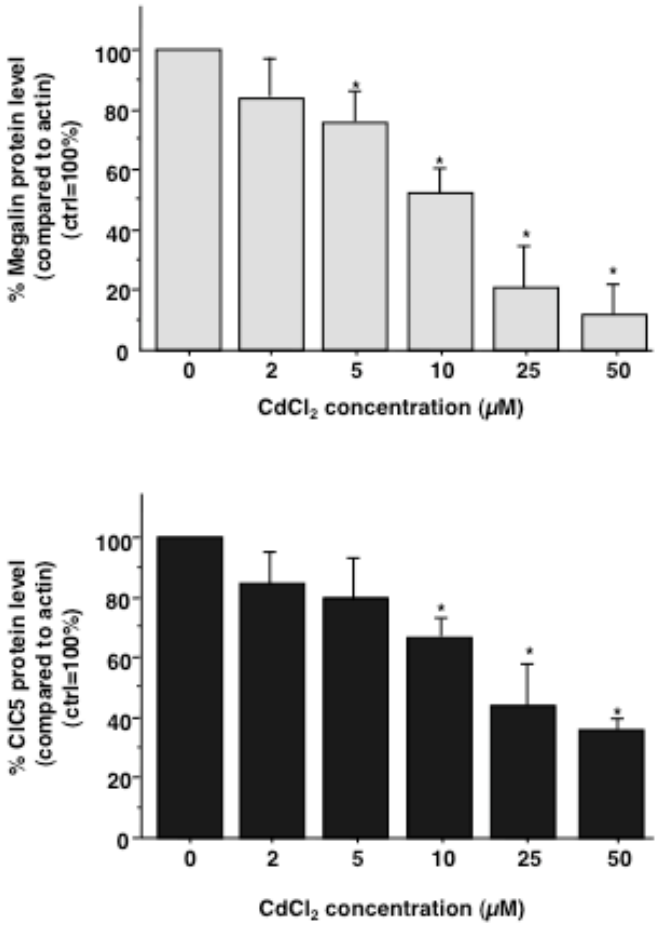
Primers Name	***Primers Sequence (5' to 3')***
Megalin-real F	CTGCTCTTGTAGACCTGGGTTC
Megalin-real R	TCGGCACAGGTACACTCATAAC
ClC5-real F	TACCCTTCGCTCCATCAACCCATT
ClC5-real R	ATGAAGGGCACGAGCTCAAAGAGA
Grp78-real F	ATCCTGGTGTTTGATCTGGGTGGT
Grp78-real R	ACACGCTGGTCAAAGTCTTCTCCA
Aqp1-real F	TGGCCAGCGAGTTCAAGAAGAAGA
Aqp1-real R	CACCTGAAGTCTGGTTGTTCCTCA
ß-act-real F	AGATGTGGATCAGCAAGCAGGAGT
ß -act-real R	TGGAATGCAACTAACAGTCCGCCT

Supplemental Material, Figure 1.



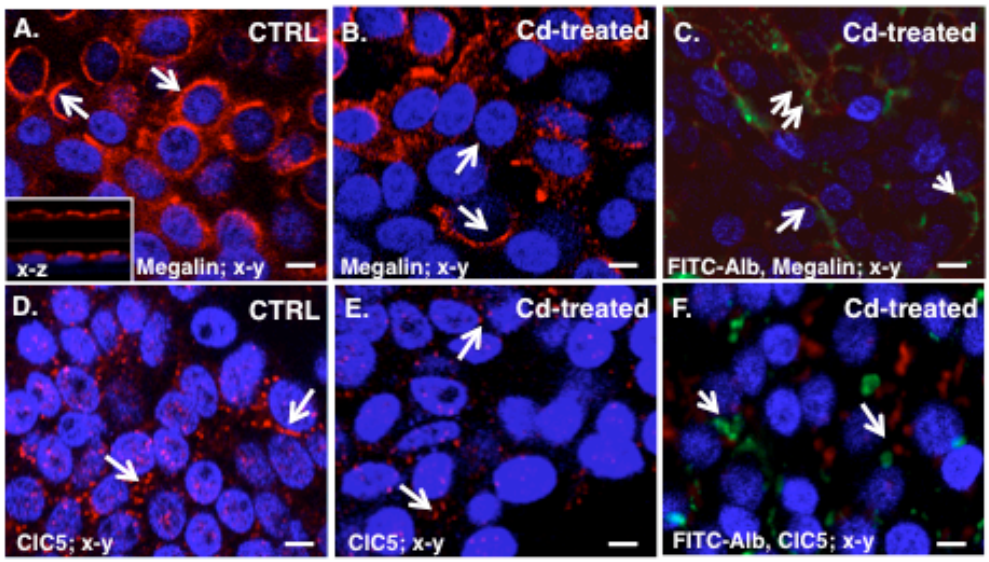
Supplemental Material, Figure 1. Concentration-response citotoxicity of $CdCl_2$ to LLC-PK1 cells assessed by MTT and LDH assays. Citotoxicity of Cd to LLC-PK1 cells was determined by the MTT (*black line*) and LDH (*gray line*) assays as reported in *Materials and Methods*. Cells were exposed to the indicated concentrations of $CdCl_2$ for 9 hours. Data shown are mean ± SE from three separate sets of experiments. Each experiment was made in triplicate. *$p < 0.05$ compared with untreated controls.

Supplemental Material, Figure 2.



Supplemental Material, Figure 2. Cadmium dose effect on megalin and CIC5 proteins. A,B. Histograms show the cadmium dose-dependence of the megalin and ClC5 protein levels. Proximal tubule cells were challenged for 9 h with 0, 2, 5, 10, 25 or 50 μM $CdCl_2$. Megalin appears more sensitive to $CdCl_2$ than ClC5. The control value (absence of $CdCl_2$) was set to 100%. Values are mean ± SE of four experiments. *$p < 0.05$ compared with untreated controls.

Supplemental Material, Figure 3.



Supplemental Material, Figure 3. Altered immunocytochemical distribution of megalin and ClC5 featuring Cd-treated cells. A,D. In LLC-PK_1 control cells, megalin (*red*) is seen predominantly on the apical membrane (*inset*) as well as in the intracellular compartment (*arrowhead*) whereas the punctuate ClC5 staining (arrows, *red*) is detected in the intracellular vesicles, particularly distributed in the perinuclear region. B,E. $CdCl_2$ exposure reduces considerably the immunoreactivity of both proteins in proximal tubule cells compared to the control ones (*arrows*). C,F. Confocal images of labeling of FITC-albumin (*green*) and megalin (*red*) or ClC5 (*red*) in $CdCl_2$-treated cells show the simultaneous decrease of FITC-albumin labeling and the immunoreactivity of the megalin and ClC5 known to be involved in the albumin endocytic process. Bars, 20 μm.